Immune Receptor NKG2D Moonlights as an Oncoprotein in Human Cancer

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The immune system is important for detecting and eliminating cancerous cells. NKG2D is a receptor found on lymphocytes, and interacts with a variety of ligands that are absent from normal cells but expressed on essentially all types of cancer cells. Upon ligand binding, NKG2D interacts with the signaling adaptor protein DAP10, which in turn activates intracellular signaling cascades that promote immune cell proliferation, survival, and cellular activities needed to destroy cancer cells. However, cancer cells have evolved multiple mechanisms to evade immune detection by this receptor, and therapeutic efforts to restore the immune response to these cancers are underway. Recent findings from the Spies laboratory and collaborators in the Clinical Research Division, however, suggest that these efforts may be misguided. As reported in *Oncogene*, the researchers found that some cancer cells co-opt expression of the NKG2D receptor, thus using the presence of NKG2D ligands for their own benefit, self-stimulating oncogenic signaling pathways and promoting tumor development.

Previous studies by the Spies group found expression of NKG2D and DAP10 on cancer cells, including breast, ovarian, prostate, and colon cancer, that could bind ligands found on the same cancer cells to activate oncogenic signaling pathways *in vitro*. The authors also positively correlated the expression of NKG2D with tumor size and spread for breast, ovarian, prostate, and colon cancer in clinical studies (Benitez *et al*., 2011). However, no study had directly examined the significance of this pathway for tumor development *in vivo*. Since spontaneous cancers in mice lack NKG2D expression, the Spies group tested *in vivo*tumorigenicity of two NKG2D and NKG2D-ligand expressing human breast cancer lines in orthotopic xenotransplant experiments. "These models showed that cancer cell NKG2D does have oncoprotein potency *in vivo,* although NKG2D effects on tumorigenesis were model specific," according to Dr. Veronika Groh.

The researchers first examined NKG2D-DAP10 transfectants of human breast cancer cell line MCF-7. MCF-7 cells express the NKG2D ligands MICA, MICB, ULBP1 and ULBP3 and were previously shown to activate oncogenic signaling cascades upon introducing NKG2D and DAP10 expression (Benitez *et al*., 2011). The incidence and time to develop tumors was significantly shorter in mice transplanted with MCF-7 cells expressing NKG2D-DAP10 versus control cells early in tumor

development (see figure). However, the transplanted tumor cells lost NKG2D expression over time, so long-term NKG2D effects on tumorigenesis could not be tested.

The researchers thus established another breast cancer cell line model, SUM149PT cells that express the ligands MICA, MICB and ULBP4, to express NKG2D-DAP10 (SUM149PT-TF). Distinct from the MCF-7 model, latency and tumor incidence were minimally changed between SUM149PT-TF and control mice. Tumor growth, however, was enhanced as determined by tumor volume. Since tumor growth is influenced by tumor cell proliferation, survival, and enhanced blood supply through angiogenesis, the researchers examined these properties in the NKG2D-DAP10 tumors versus the control tumors. While the SUM149PT-TF cells in culture showed enhanced cell cycle progression and increased metabolism, *in vivo* the proliferation or survival of cells in the tumors was not changed. However, the researchers found more blood vessels in the SUM149PT-TF tumors compared to control tumors, and in culture, these cells secreted more vascular endothelial growth factor (which promotes new blood vessel formation). They also found instances of tumor cells present in the blood vessels, as well as small clumps of tumor cells within the lungs of SUM149PT-TF mice indicative of micrometastases.

Overall, the authors conclude that NKG2D has the ability to promote tumorigenicity *in vivo* through self-stimulated activation of oncogenic signals within cancer cells. Depending on the model used, NKG2D affects either: 1) tumor initiation; or 2) growth and spread through enhanced angiogenesis. According to the Dr. Groh, these results "have clinical relevance as lymphocyte NKG2D and its ligands are currently targeted for cancer immunotherapy. These approaches, however, do not account for effects of cancer cell NKG2D as an oncoprotein and may thus be misdirected."

El-Gazzar A, Cai X, Reeves RS, Dai Z, Caballero-Benitez A, McDonald DL, Vazquez J, Gooley TA, Sale GE, Spies T, Groh V. 2013. Effects on tumor development and metastatic dissemination by the NKG2D lymphocyte receptor expressed on cancer cells. *Oncogene.* Epub ahead of print; doi: 10.1038/onc.2013.435.

See also: Benitez AC, Dai Z, Mann HH, Reeves RS, Margineantu DH, Gooley TA, Groh V, Spies T. 2011. Expression, signaling proficiency, and stimulatory function of the NKG2D lymphocyte receptor in human cancer cells. *Proceedings of the National Academy of Science USA* 108:4081-4086.

El-Gazzar A, Groh V, Spies T. 2013. Immunobiology and conflicting roles of the human NKG2D lymphocyte receptor and its ligands in cancer. *Journal of Immunology* 191:1509-1515.

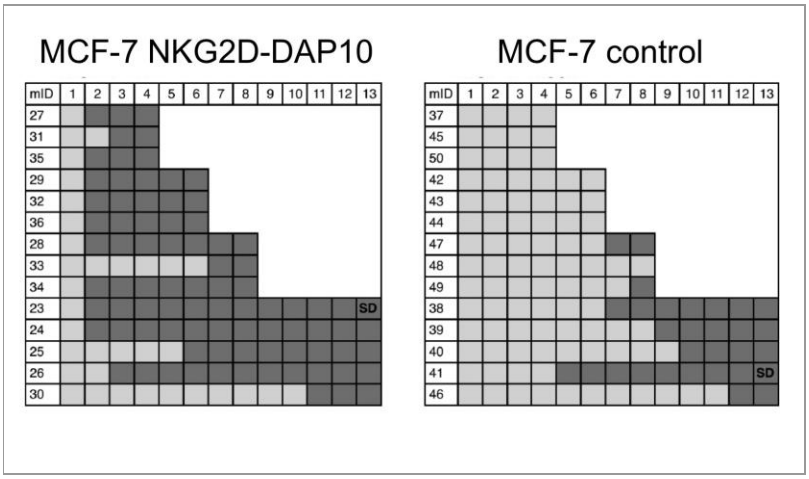

Image provided by Dr. Veronika Groh

NKG2D expression reduces latency and enhances tumor take in human breast cancer MCF-7 xenotransplant model. Graphic represents tumor development over time in weekly intervals (top numbers) for individual mice (vertical numbers). Dark boxes indicate measurable tumor mass.